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MINIREVIEW

Older but Not Wiser: the Age-Driven Changes in Neutrophil Responses during Pulmonary Infections

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ABSTRACT Elderly individuals are at increased risk of life-threatening pulmonary infections. Neutrophils are a key determinant of the disease course of pathogen-induced pneumonia. Optimal host defense balances initial robust pulmonary neutrophil responses to control pathogen numbers, ultimately followed by the resolution of inflammation to prevent pulmonary damage. Recent evidence suggests that phenotypic and functional heterogeneity in neutrophils impacts host resistance to pulmonary pathogens. Apart from their apparent role in innate immunity, neutrophils also orchestrate subsequent adaptive immune responses during infection. Thus, the outcome of pulmonary infections can be shaped by neutrophils. This review summarizes the age-driven impairment of neutrophil responses and the contribution of these cells to the susceptibility of the elderly to pneumonia. We describe how aging is accompanied by changes in neutrophil recruitment, resolution, and function. We discuss how systemic and local changes alter the neutrophil phenotype in aged hosts. We highlight the gap in knowledge of whether these changes in neutrophils also contribute to the decline in adaptive immunity seen with age. We further detail the factors that drive dysregulated neutrophil responses in the elderly and the pathways that may be targeted to rebalance neutrophil activity and boost host resistance to pulmonary infections.

KEYWORDS neutrophils, aging, pneumonia, lung infections, immunosenescence

The population of elderly individuals is on the rise in the United States. Estimates are that 21.7% of the population will be 65 years old or older by 2040 (1). The increased susceptibility to pulmonary infections as we age poses a serious risk to longevity and the quality of life (2). The incidence of community-acquired pneumonia (CAP) is significantly higher in the elderly than in younger adults and is associated with increased mortality, longer time to recovery, and extended hospital stays (3, 4). In fact, approximately 60% of hospital admissions of older adults are estimated to be due to pneumonia (5), and both bacterial and viral infections are the leading causes of CAP in the elderly (6). The Gram-positive bacterium *Streptococcus pneumoniae* (pneumococcus) remains the most frequent bacterial agent of CAP in individuals over the age of 65 years (6), but other bacterial agents associated with CAP in the elderly include *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, and *Legionella* species (6). In addition, prior to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, the most common viral agents associated with CAP in the elderly were rhinoviruses and influenza viruses, followed by respiratory syncytial virus and parainfluenza viruses (6). Polymicrobial infections are also commonly detected, with bacterial-viral combinations found in >30% of cases (6).

The role of PMNs in pneumonia. Successful control of a pulmonary infection is initially carried out by the host's innate immune response, particularly by polymorphonuclear cells (PMNs), also known as neutrophils (7). PMNs are relatively short-lived cells that are continuously produced in the bone marrow in large numbers (10^{11}/day) (7). Circulating PMNs patrol the body for signs of infection and, in response to local

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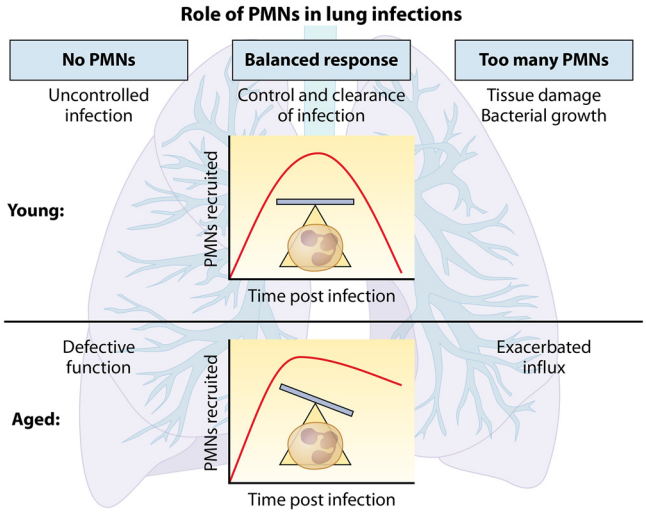

FIG 1 Aging is associated with dysregulated PMN responses. In young hosts, a balanced neutrophil response, where neutrophils migrate to the site of infection, perform antimicrobial functions, and then resolve, is required for proper control and clearance of a pulmonary infection. However, aged hosts experience a decline in PMN function, exacerbated PMN pulmonary influx, and impaired resolution. This unbalanced response results in microbial persistence, tissue damage, and worse disease outcomes.

inflammation, are among the first cells to be recruited to the site of infection to control invading pulmonary pathogens (7) (Fig. 1). Neutropenic individuals, or those with impaired PMN antimicrobial activity, are at increased risk for pneumonia (7–9). In animal models, depletion of PMNs prior to several bacterial (10–13) or viral (14, 15) pulmonary infections results in increased pathogen burdens and heightened mortality. These findings emphasize the importance of PMNs early on in controlling pathogen burdens (Fig. 1). Importantly, however, an exacerbated neutrophilic response can also contribute significantly to lung pathology, because PMNs can inflict major damage on the host (7, 16). In fact, impairment of PMN resolution in the lungs is linked to poorer outcomes, and depletion of these cells after the onset of disease enhances host survival in several models of bacterial and viral pulmonary infections (10, 12, 15). Therefore, successful control of pulmonary infections requires a balance of efficient clearance of invading pathogens by PMNs with the subsequent resolution of these immune cells to prevent tissue damage (Fig. 1).

In this review, we discuss the role of PMNs in the susceptibility of the elderly to pneumonia. We first summarize the age-driven changes in PMN recruitment, resolution, and antimicrobial function (Fig. 1). We highlight the role of PMNs beyond innate immunity and point to the need for further studies to determine whether age-driven changes in PMNs also contribute to the decline in adaptive immunity seen in aging hosts. We conclude by discussing why PMN responses are dysregulated with aging and what pathways may be targeted to rebalance these responses and boost resistance to pulmonary infections (Fig. 2).

AGE-DRIVEN CHANGES IN NEUTROPHIL RESPONSES

Aging is accompanied by persistent low-grade inflammation, known as inflammaging, which, in turn, drives immunosenescence, i.e., the overall decline in immunity seen in the elderly (17). Both phenomena contribute to increased susceptibility of the elderly to pulmonary infections (18, 19). Although PMNs have a shorter life span than other immune cells and are continuously produced in the bone marrow, these innate immune cells also display age-driven impairment (20) (Fig. 1 and 2). However, unlike that of other immune cells. such as naive T cells, the production of PMNs is not diminished in the elderly, who, if healthy, have equivalent or greater numbers of circulating PMNs than

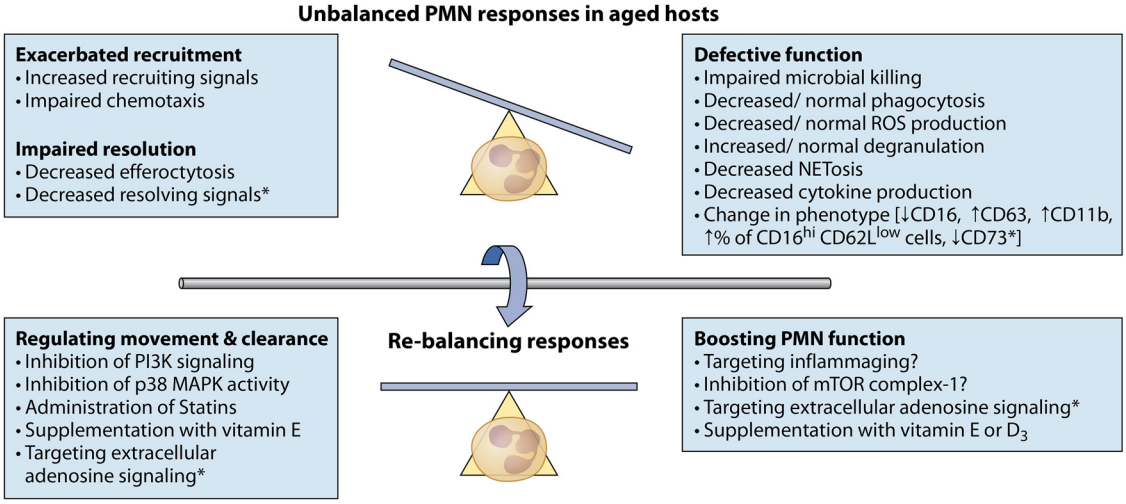

FIG 2 Approaches to rebalancing PMN responses in aged hosts. Several potential pathways and targets have been identified that may boost neutrophil antimicrobial activity, regulate PMN migration, and improve host resistance to infection. Asterisks indicate studies performed only in mice.

younger controls (21–23). Instead, PMN impairment is driven by intrinsic changes, as well as changes in the microenvironment, as discussed below.

In accord with the term "polymorphonuclear," mature PMNs are characterized by segmented nuclei. These cells differ considerably among species, however, in terms of both their antimicrobial responses and the markers they express. For example, mouse neutrophils express high levels of Ly6G on their surfaces, but Ly6G is not expressed in humans. Rather, human PMNs are mostly characterized by high forward/side scatter and expression of CD16, CD15, or CD66b (24). Since murine models do reflect the age-driven susceptibility of humans to pulmonary infections (15, 25–27), we will focus here on PMN responses from human donors and discuss mouse models of pneumonia that support these findings.

Recruitment. To clear pathogens efficiently, PMNs must reach the site of infection (Fig. 1). In response to microbial challenge, resident pulmonary cells, including alveolar macrophages and lung epithelial cells, produce cytokines and chemokines that recruit PMNs (28). Recruited PMNs move from the circulation across the endothelium, enter the interstitial space, and then cross the lung epithelium into the airways (28). This process involves a complex network of ligand/receptor interactions, as well as cytokine and chemokine receptor signaling (reviewed elsewhere [28]). In aged hosts, general PMN recruitment into the lungs is actually elevated (15, 25, 29–31), but the accuracy of PMN trafficking toward the pulmonary insult is impaired (27, 32, 33) (Fig. 2). With aging, uninfected healthy human donors have elevated PMN levels in the airways; the percentage and number of PMNs in bronchoalveolar lavage fluid (BALF) are significantly higher than those in young controls (34–36). This elevation may be driven by inflammaging, since the BALF of elderly donors contains increased levels of cytokines that mediate PMN recruitment, such as interleukin 6 (IL-6) and IL-8 (35, 36). Although very few studies have examined PMN pulmonary influx during pneumonia in elderly individuals, elderly patients with pneumococcal pneumonia exhibit higher percentages of neutrophils in lung biopsy specimens than younger patients (29). While PMNs from healthy elderly volunteers do not display any defects in endothelial adherence (37) or transepithelial migration in response to pneumococcal infection *in vitro* (38), the chemotaxis (directional movement) of PMNs is impaired in aging (32, 33). This impairment is independent of local signaling; instead, it is driven by intrinsic elevated basal levels of phosphoinositide-3-kinase (PI3K) signaling (32). PMNs isolated from healthy elderly donors show reduced chemotaxis toward sputum from *S. pneumoniae* patients relative to that for younger donors (32), and this age-driven impairment in migration is even

more pronounced in circulating PMNs isolated from elderly pneumonia patients, worsening progressively with increased disease severity (33). While chemotaxis toward the stimulus is reduced, chemokinesis, or random movement, of PMNs from elderly donors is not impaired (32). Thus, PMN migration in the elderly is inaccurate, i.e., it does not follow a trajectory toward the stimulus, which may impair the ability of PMNs to efficiently reach the site of infection. This dysregulated movement may contribute to lung damage (Fig. 1), because PMNs from elderly donors release more neutrophil elastase (32, 38). When present in excess, neutrophil elastase can damage pulmonary epithelial cells and vasculature (39), blunt the immune responses of macrophages (40), and impair host resistance to pneumococcal pneumonia, as seen in mouse models (40). Therefore, although aging does not lead to impairment of pulmonary PMN recruitment, PMN colocalization with the pathogen (27), which is necessary for efficient pathogen control and mitigation of bystander damage to tissues, is impaired (Fig. 2).

Resolution. Successful control of a pulmonary infection requires efficient clearance of invading pathogens by immune cells, followed by the resolution of inflammation to prevent tissue damage (7) (Fig. 1). However, aging is associated with an enhanced PMN presence in the lungs in response to pulmonary infections (Fig. 2), observed both in humans (29) and in several mouse models of bacterial and viral pneumonia. PMN numbers in the lungs of old mice are significantly higher than those in young controls during pulmonary infection with *Acinetobacter baumannii* (31), *S. pneumoniae* (25, 30), or influenza A virus (IAV) (15). Following *S. pneumoniae* (10) or IAV infection (41), the numbers of PMNs from the lungs of young mice peak and then resolve over time, but these kinetics are dysregulated with aging, i.e., resolution of pulmonary inflammation is not observed in old mice (15, 25, 30, 41) (Fig. 1). As discussed above, PMN persistence can cause lung damage, whereas experimental depletion of PMNs in the later phase of disease following IAV infection enhances the survival of aged mice without altering the pathogen burden (15). These findings suggest that PMN persistence in the pulmonary space is pathogenic in aged hosts (Fig. 1).

PMN resolution from the lungs is an active process that involves the clearance of apoptotic PMNs present in the pulmonary environment, abrogation of signals that mediate the recruitment of new cells, and production of mediators that negatively regulate PMN influx (42). These factors have been characterized mostly in animal models. One factor that apparently contributes to exacerbated PMN pulmonary presence is the inability of aged hosts to control pathogen burdens. Persistent pathogen detection by resident pulmonary cells leads to the continuous release of inflammatory signals that recruit more cells into the lungs. For example, pneumococcal pneumonia in aged mice results in increased bacterial numbers that correlate with elevated production of inflammatory cytokines that recruit PMNs (25, 30, 43, 44). Similarly, elderly human patients show extended inflammation following pneumococcal infection (45).

PMNs are relatively short-lived cells that undergo spontaneous apoptosis. However, upon PMN entry into inflamed tissue, apoptosis is delayed by inflammatory cytokine signaling in the milieu, allowing PMNs to perform their antibacterial function before death and clearance by resident macrophages. Aging is not accompanied by impaired apoptosis; rather, the ability of inflammatory signals to delay PMN apoptosis is blunted (46, 47). This possibly contributes to the reduced ability of PMNs in aged hosts to control pathogen burdens (Fig. 2) (discussed in the next section). The ability of macrophages to perform efferocytosis, i.e., the engulfment and clearance of apoptotic cells, is impaired with aging (42). In IAV infection of the lungs of aged mice, both the number of alveolar macrophages and their ability to phagocytose apoptotic PMNs are reduced, resulting in impaired PMN resolution (48). One factor, resolvin D1, may contribute to this reduced macrophage efferocytosis. Resolvins belong to a family of specialized proresolving lipid mediators that are crucial for the resolution of inflammation in several organs, including the lungs (49). In mouse models of pulmonary *Escherichia coli* (50) or *Pseudomonas aeruginosa* (51) infection, or in secondary pneumococcal pneumonia (52), resolvin D1 is crucial for host survival and the reduction of PMN influx into the

lungs, as well as for PMN clearance by macrophages. Interestingly, the production of resolvin D1 is reduced in aging (53), and treatment of acute sterile lung injury with this lipid mediator boosts macrophage efferocytosis and ameliorates lung damage in aged mice (54). The role of resolvins in exacerbated PMN responses in elderly individuals during lung infection requires further elucidation.

Extracellular adenosine (EAD) is another regulator of pulmonary PMN influx. Upon tissue damage by infection or a variety of other insults, ATP is thought to leak from damaged cells into the extracellular space, where it can be metabolized to adenosine by the sequential action of two extracellular enzymes: CD39, which converts ATP to AMP, and CD73, which dephosphorylates AMP to EAD (55). EAD can then bind to, and signal via, four G-protein-coupled receptors (56) that are ubiquitously expressed (57). EAD receptor signaling may enhance or diminish acute inflammation, depending on the target receptor, cell type, and/or EAD concentration (56, 57). This pathway has been characterized in several models of sterile inflammation, although its role in pulmonary infections has only recently been appreciated (58–61). In sterile lung injury models, CD73 is important for PMN resolution (62, 63), and EAD production by CD73 has been found both to blunt the transendothelial migration of PMNs into the lung interstitium during pneumococcal pneumonia and to play an essential role in PMN resolution and the prevention of lung damage (10). Similarly, CD73 blunts PMN pulmonary influx during *Mycobacterium tuberculosis* infection (64). Interestingly, CD73 expression on PMNs and EAD production by PMNs in response to *S. pneumoniae* infection are reduced in aged mice (65). This suggests that the EAD pathway is dysregulated during aging and that this dysregulation may contribute to the deficiency in PMN resolution during pulmonary infection.

In summary, several factors have been shown to contribute to the dysregulated PMN responses observed during lung infections of aged hosts (Fig. 2). Elucidating the pathways controlling PMN responses and how they are altered by aging is crucial for devising means to control the regulation of PMN movement and to boost host resistance, as discussed below.

Antimicrobial activity. PMNs kill bacteria by several mechanisms (66). Antimicrobial factors packaged in primary, secondary, and tertiary granules are formed sequentially in PMNs during cellular differentiation in the bone marrow and are released upon encountering a pathogen (67). More-recent findings also show that active transcription is crucial for PMN antimicrobial function, and transcriptional profiling has been used to characterize changes in PMN responses upon influx into the lungs during infection (68, 69). The mechanisms by which PMNs kill microbes include phagocytosis and intracellular killing via the fusion of granules with the phagosome, as well as intracellular production of reactive oxygen species (ROS) (66). PMNs can also kill microbes extracellularly, via degranulation and release of antimicrobial contents, extracellular production of ROS, and the formation of neutrophil extracellular traps (NETs) (66). Many studies have characterized the responses of human PMNs upon *ex vivo* stimulation, revealing that aging is accompanied by overall declines in phagocytosis (70–73), ROS production in response to stimuli (71, 73–75), production of NETs (NETosis) (76), and cytokine production (77, 78) (Fig. 2). However, several studies failed to find age-associated differences in phagocytosis or the oxidative burst (21, 75, 79, 80) (Fig. 2). Fewer studies have examined degranulation; they found either no age-related difference upon stimulation (21, 79) or an increase in the amount of released neutrophil elastase (38), a component of primary granules (Fig. 2). These discrepancies may be driven by several factors, as outlined below.

First, the heterogeneous nature of the elderly, driven by underlying conditions, infections, medications, etc., can confound results when one is studying immune responses (81). To circumvent this issue, the SENIEUR protocol, with very strict inclusion and exclusion criteria, was implemented in the 1980s to assess the immune responses of healthy elderly donors (82). However, only a small percentage (~10%) of elderly people actually satisfy these criteria, and they may not include the individuals at increased risk of infection (81). Interestingly, the immune responses of healthy elderly individuals might contribute to longevity. A study comparing neutrophil

responses across age groups found that the PMN responses of healthy centenarians, i.e., people 100 years of age or older, are comparable to those of young adults with respect to ROS production and phagocytosis, while the same PMN responses are blunted in younger elderly donors (aged 65 to 75 years) (73). In addition, there is a well-known sex-based difference in host susceptibility to infections, as reflected in the incidence of invasive pneumococcal pneumonia, which is higher in males than in females, regardless of age (83, 84). Furthermore, some studies have found that the effect of age on PMN responses is more pronounced in males than in females (73, 85).

A second factor that may confound results is the immune stimulant used. Many studies use either inert beads, bacterial products such as *N*-formylmethionyl-leucyl-phenylalanine (FMLP) formulated peptides and lipopolysaccharides (LPS), or chemicals such as phorbol 12-myristate 13-acetate (PMA) (70–78), while much fewer studies have characterized the age-driven changes in PMN-pathogen interactions (38, 70, 71). While the compounds mentioned above are potent PMN stimulants, they may not always accurately reflect the immune responses to actual pathogens. It is well known that pathogens modulate PMN activities (86), whereas microbial products may not do so accurately. For example, while FMLP inconsistently reveals differences in ROS production across studies (21, 75, 79, 80), ROS production in response to bacterial pathogens, including *S. aureus* (71) and *S. pneumoniae* (87), has consistently been shown to be altered in aging. This response is also pathogen specific. In one study, whereas ROS production by PMNs in response to *S. aureus* was reduced in elderly donors from that in young controls, ROS production in response to *E. coli* was constant across age groups (71). Overall, however, with regard to PMN responses to actual microbes, there is an age-related decline in phagocytosis and intracellular killing of engulfed pathogens, as demonstrated for *Candida albicans* (88), *E. coli*, *S. aureus* (71), and *S. pneumoniae* (70).

The manner in which PMNs "see" a pathogen is a third factor that can influence PMN responses. Serum complement and antibodies can mediate opsonophagocytic uptake and killing of bacteria via complement receptors or antibody (Ab) Fcγ receptors (FcγR) expressed on PMNs (89). Several studies have revealed that antibody-mediated PMN responses diminish with age (70, 85). For example, *S. pneumoniae* opsonized with Ab-containing serum from an immunized young donor is killed less efficiently by PMNs from elderly donors than by PMNs from young donors (70). This age-related decline in antibody-mediated killing (85) may be related to a decline in FcγRIII (CD16) expression on PMNs (72). In contrast, complement-mediated PMN responses are not impaired with aging. In fact, serum complement activity and the expression of complement receptors on PMNs either remain unchanged or increase with aging (70, 90). Similarly, the ability of PMNs from elderly donors to kill *S. pneumoniae* preopsonized with rabbit complement is actually higher than that for young controls (38). In contrast, opsonization of bacteria with matching serum from each donor consistently demonstrates impaired pathogen killing by PMNs from elderly volunteers (70, 71). These findings suggest that conditions that closely mimic *in vivo* environments (found in the host) more accurately reveal an age-driven decline in pathogen control. In summary, it is important to keep in mind that several aspects of study design can confound the responses of human donors and may mask important age-driven changes in PMN function.

In mouse models, aging is more consistently associated with impaired PMN antibacterial activity. During *S. aureus* skin infections, blunted NETosis is linked to the spread of systemic infection in aged mice (91). In the case of *A. baumannii* pneumonia, aged mice have higher pulmonary CFU counts and lower survival rates than young controls, a finding linked to both reduced release of myeloperoxidase (a component of primary granules) and reduced ROS production by pulmonary PMNs (31). Aging also impairs the ability of murine PMNs to kill engulfed *S. pneumoniae* (65). Importantly, adoptive transfer of PMNs from young controls boosts the resistance of aged mice to pneumococcal pneumonia, highlighting the importance of these cells in host resistance to pulmonary infection (65). While several changes in PMN effector activities in old versus young mice have been described (Fig. 2), the signaling pathways driving these

changes are less well characterized. Elucidating these pathways will be key for improving PMN antimicrobial function, as discussed in "Approaches for reversing dysregulated PMN responses in aging" below.

NEUTROPHIL PHENOTYPES IN AGED HOSTS

Originally thought to be homogeneous, PMNs differ in their expression of surface markers, in their nuclei, and in their density and activity (24, 92, 93). This heterogeneity impacts PMN phenotypes and antibacterial function, as seen in certain disease states and during pulmonary infection (24, 93). The factors that drive PMN heterogeneity remain unclear. During persistent infection and systemic inflammation, the increased demand for PMNs triggers emergency granulopoiesis, characterized by the mobilization of immature precursors from the bone marrow, resulting in the presence of both mature and immature PMNs with distinct characteristics in the circulation (94). Further, direct interaction with bacteria, as well as local reprograming within the lungs, can alter the phenotypes of PMNs and their ability to kill and clear pathogens (24, 92, 93). However, it remains unclear whether there are bona fide PMN subsets that arise from specific progenitors, and this question is currently being investigated (24, 92, 93). The concept of PMN heterogeneity, the classification of PMN phenotypes, and the question of whether heterogeneity is a result of true subsets, a reflection of different maturation states, or a response to the microenvironment have been extensively reviewed elsewhere (24, 92, 93). Less is currently known about the spectrum of PMN phenotypes during host aging (Fig. 2).

Changes in PMNs driven by infection. Circulating PMNs, which have a higher density than peripheral blood mononuclear cells (PBMCs) (95), are typically isolated via density gradient centrifugation. However, in patients with active *Mycobacterium tuberculosis* infection, lower-density neutrophils (LDNs) that localize with the PBMC fraction are found in the circulation (96). These cells are thought to result from bacterial activation of mature PMNs and to provide an intracellular niche for bacterial replication (96). During pathological conditions, a population of myeloid-derived suppressor cells (MDSCs) that display granulocytic markers and have T cell suppressor functions are also found within the PBMC fraction (97). These granulocytic MDSCs are proposed to be a population of suppressive PMNs that inhibit T cells (97). In humans, granulocytic MDSCs are found in the circulation of *M. tuberculosis* patients with active infection (98) and in cystic fibrosis (CF) patients with *P. aeruginosa* infections (99). In addition, incubation of peripheral blood with bacteria results in the formation of granulocytic MDSCs, suggesting that microbial products can also cause the generation of these cells (99). Importantly, both LDNs and granulocytic MDSCs are associated with more-severe infections and worse disease outcomes (96, 98, 99). In mouse models, granulocytic MDSCs also enhance disease progression during pulmonary infections (100, 101). Aging is associated with an overall expansion of MDSCs (102); however, it is unclear whether granulocytic MDSCs or other LDNs are also altered with aging.

The extracellular-adenosine-producing enzyme CD73 is required for the ability of murine PMNs to kill pneumococci (103). In a mouse model of *S. pneumoniae* lung infection, PMNs recruited into the lungs early on express CD73, which correlates with bacterial control (103). However, as infection progresses, $CD73^{low}$ PMNs accumulate in the lungs and are associated with enhanced bacterial infection (103). $CD73^{low}$ PMNs express lower levels of Ly6G, possibly indicating a less-mature PMN subset. $CD73^{low}$ PMNs are also found in aged mice (65), in which circulating PMNs express lower levels of CD73 than those of young controls, impairing their ability to kill *S. pneumoniae* (65). In humans, PMNs may express CD73 intracellularly (104) rather than on their surfaces (105). Extracellular adenosine signaling is known to play a role in human PMN apoptosis (106), phagocytosis (107), ROS production (108), and degranulation (109), in response to the presence of bacterial products (57). Further, CD73 activity is required for *S. pneumoniae* killing by PMNs in ~60% of young donors (103), suggesting that the expression of this enzyme may also differ among individuals.

Changes in PMNs driven by inflammation. PMNs with distinct phenotypes appear in the circulation during inflammation and can be characterized in whole blood using flow cytometry in the absence of density gradient centrifugation (110). In healthy young volunteers receiving intravenous injections of LPS, three PMN populations, differentiated by their nuclei as well as by CD16 and CD62 ligand (CD62L) expression, appear within hours. The populations consist of segmented mature $CD16^{hi}$ $CD62L^{hi}$ PMNs, immature $CD16^{low}$ $CD62L^{hi}$ band cells, and hypersegmented $CD16^{hi}$ $CD62L^{low}$ cells (110). These cells differ in function; $CD16^{hi}$ $CD62L^{low}$ cells are suppressive PMNs that inhibit T-cell proliferation (110, 111). Further, the populations differ in antibacterial activity: $CD16^{low}$ $CD62L^{hi}$ band cells are efficient at killing *Staphylococcus aureus*, while $CD16^{hi}$ $CD62L^{low}$ cells have impaired antibacterial function. Similar surface changes in circulating PMNs are observed in children with severe viral pneumonia (112), where $CD16^{hi}$ $CD62L^{low}$ PMNs are present in the airways of patients with bacterial coinfections, suggesting that these cells can enter the lungs and possibly play a role in impaired pulmonary control of bacteria. Importantly, $CD16^{hi}$ $CD62L^{low}$ PMN levels are elevated in the circulation of elderly donors relative to those in young controls (74). The presence of these cells is thought to contribute to the impaired bacterial phagocytosis and ROS production detected in total PMNs of elderly donors (74). Inflammaging, characterized by increased basal levels of circulating inflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNF-α) (17, 80), might contribute to the phenotypic and functional changes in PMNs in elderly donors as well. The circulation of frail elderly donors shows an increase in the presence of activated immature band cells, with increased spontaneous ROS production and elevated expression of CD11b. Elevated CD11b expression correlates with increased levels of TNF-α in the sera of donors (87). In mice, genetic abrogation of TNF-α has been shown to prevent this age-driven increase in CD11b expression on PMNs (87). These data suggest that the inflammatory environment in the circulation of elderly donors may play a role in altering the phenotypes and functions of PMNs and thus could be a potential target for combatting immunosenescence (Fig. 2).

Changes in PMNs driven by the pulmonary environment. Migration of PMNs from the circulation into tissues results in their activation and potentiation of their antimicrobial activity (7, 113). However, under certain disease conditions, the pulmonary microenvironment can cause aberrant changes in PMNs, resulting in impaired function. This has been described in cystic fibrosis patients (114) and in children with bacterial-viral coinfections (115) and can be modeled *in vitro* using a transwell system seeded with lung epithelial cells. It has been found that migration of PMNs from healthy donors across lung epithelial cells in response to airway fluid supernatant from either cystic fibrosis patients (116) or children with bacterial-viral coinfections (115) resulted in a loss of CD16 (an Fcγ receptor involved in phagocytosis) and an increase in the expression of CD63 (a marker of primary degranulation). These changes are associated with an increase in neutrophil elastase in the airway fluids. Importantly, these migrating PMNs displayed defects in their abilities to kill *P. aeruginosa* (116), *H. influenzae* (115), and *S. aureus* (115). In separate studies (32, 72), PMNs from elderly donors have also been shown to express lower levels of CD16 and elevated levels of CD63. Intriguingly, age-driven changes in the pulmonary microenvironment, including changes in pulmonary epithelial cells and increased levels of neutrophil elastase, are known to occur (35, 36, 117). Thus, age-related changes in the lungs may further contribute to the age-driven PMN impairment seen in pneumonia, suggesting that the effect of the pulmonary microenvironment on PMN antibacterial function in aged hosts is an important area for further investigation.

In summary, changes in the circulation and in the pulmonary environment during aging may contribute to an alteration in PMN responses (Fig. 2). Further characterization of the age-driven changes in PMN phenotype and function, the mechanisms driving these changes, and their link to the susceptibility of the elderly to pneumonia is

needed in order to understand these processes more fully and potentially identify relevant drug targets.

THE ROLE OF NEUTROPHILS BEYOND INNATE IMMUNITY

Apart from their role in innate immunity, recent studies have highlighted the role of PMNs in orchestrating adaptive immune responses (118). PMNs can act as antigen-presenting cells (APCs), directly interact with B cells to induce Ab production, and activate or suppress T-cell responses (118). In some instances, these activities are mediated by PMNs with special characteristics (92). Although aging is accompanied by a well-characterized decline in B- and T-cell responses (119), little is known about how the age-driven alteration of PMN responses may contribute to the decline in adaptive immunity.

Interaction of PMNs with DCs. PMNs can mediate dendritic cell (DC) recruitment and maturation (120). Several bacteria induce murine PMNs to produce chemokines that recruit DCs (120). Human PMNs can also produce chemokines when stimulated *ex vivo*, but interestingly, chemokine production by PMNs from elderly donors is diminished (77, 78). During pulmonary infections, PMNs in the lungs are also important for instructing DCs to migrate to the lung-draining lymph nodes, where they activate T cells (121, 122). For several pulmonary viral infections, including IAV infections, DC activation and trafficking to the draining lymph nodes are diminished in aged mice (41, 123), and early PMN recruitment to the lungs is delayed (41). Therefore, it is possible that changes in PMNs contribute to the impaired DC responses described in aged hosts.

Interaction of PMNs with T cells. PMNs produce a repertoire of chemokines that can recruit T cells to the site of inflammation (124). During IAV infection in mice, PMNs produce chemokine trails that guide $CD8^+$ T cells into the trachea (125). Aged mice show a delay in $CD8^+$ T-cell recruitment into the lungs (41, 123), but it is unclear whether age-related changes in PMN responses affect the pulmonary recruitment of other immune cells following infection. PMNs can also acquire APC characteristics and present antigen to T cells. In human donors, circulating PMNs acquire DC markers during bacterial infections (126). Upon antigen exposure *ex vivo*, PMNs from young, healthy human donors upregulate major histocompatibility complex class II (MHC-II) and costimulatory molecules (127, 128) and acquire the ability to present IAV antigens to $CD4^+$ T cells (127). In mice, PMNs with DC characteristics can acquire and present antigen at the site of infection (129) and have been shown to trigger the proliferation of antigen-specific $CD4^+$ T cells during fungal pneumonia (130). Since antigen presentation by professional APCs declines with aging (81, 131), PMNs may thus play an important role in boosting T-cell responses in aged hosts. PMNs can further activate T cells by producing cytokines that can drive T-cell subset differentiation (132), and PMN-derived products, such as NETs, can further prime T cells to respond more efficiently to antigens (133). However, both PMN cytokine production and NETosis have been shown to be blunted in elderly donors (76–78).

On the other hand, PMNs can also suppress T cells, since neutrophil-derived serine proteases, such as neutrophil elastase, can cleave cytokine receptors involved in T-cell activation (134). Interestingly, PMNs from healthy elderly donors have higher neutrophil elastase activity than those from young controls (38); therefore, it is possible that changes in PMNs during aging lead to the suppression of T cells. Further, it has been proposed that granulocytic MDSCs, described above, are a population of suppressive PMNs (97) that produce an arsenal of effectors that inhibit T-cell proliferation (95), including ROS (135) and arginase-1 (136). In some studies, basal ROS production by PMNs has been reported to be elevated in elderly subjects (87), and $CD16^{hi}$ $CD62L^{low}$ cells, thought to be suppressive PMNs (110), are more numerous in the elderly (74). Thus, since aging is associated with a well-characterized decline in T-cell responses (137, 138), it is possible that an impairment of PMN responses that activate T cells or an increase in T-cell-suppressing PMNs contributes to this decline.

Interaction of PMNs with B cells. In human spleen, a subset of PMNs has been found to directly induce Ab production and class switch recombination by B cells in

response to T-cell-independent antigens (139). PMNs play a role in natural Ab responses against pneumococci, and patients with neutropenic disorders have lower levels of Abs to some bacterial strains than healthy controls (139). PMNs that mediate B-cell help are also present in mice (140) and nonhuman primates (141). In mice, PMNs localize with marginal-zone B cells in the spleen and are required for the production of T-cell-independent Abs during systemic *S. pneumoniae* infection (140, 142). Aging is associated with an overall decline in B-cell responses and reduced Ab production and functionality (143, 144). However, the contribution of PMNs to this decline remains unexplored.

Role of PMNs in vaccine responses. In addition to bridging the innate and adaptive responses, PMNs also play an important role in vaccine-mediated protection against pulmonary pathogens (140). PMNs have traditionally been viewed as effectors of vaccine responses, i.e., vaccination triggers Abs, and, as one mechanism of protection, Abs bind to extracellular bacteria and promote their clearance by enhancing uptake and killing by PMNs (70). Evidence that the age-related decline in the intrinsic microbicidal activities of PMNs may contribute to decreased pneumococcal polysaccharide vaccine (PPSV) efficacy in the elderly stems, in part, from the finding that *S. pneumoniae* opsonized with the sera of young donors immunized with PPSV are killed less efficiently by PMNs from elderly donors than by PMNs from young donors. This finding suggests that PMN function is impaired in elderly subjects, even in the presence of specific antibodies (70).

Recent evidence indicates that PMNs also induce vaccine responses in mouse models. In these models, B-helper murine PMNs, described above, are required for Ab production in response to PPSV, which elicits T-cell-independent Ab production (140). Further, PMN depletion at the time of vaccination with T-cell-dependent vaccines in mice also results in the loss of host protection against subsequent challenge (145). Depletion of PMNs during vaccination with a recombinant *M. tuberculosis* protein-based vaccine prevented mice from mounting protective Th1 and Th17 responses and prevented the reduction of pulmonary bacterial burdens in vaccinated mice (146). Similarly, in preclinical studies, PMN depletion in mice at the time of vaccination with Prevnar 13, the pneumococcal polysaccharide conjugate vaccine, resulted in the loss of protective Ab responses and a lack of host protection against subsequent *S. pneumoniae* lung infection (145). Aging leads to defects in both T-cell-dependent and T-cell-independent Ab production following the vaccination of human hosts (143, 144), limiting the efficacy of current vaccines (25, 144). However, the role of PMNs in this phenomenon in human hosts remains an open question. In nonhuman primates, vaccination elicits changes in circulating PMNs (141). Whether PMNs are also activated following human vaccination has not been tested, and it is unclear whether PMNs in the elderly fail to acquire an APC-like provaccine phenotype. Interestingly, although the expression of HLA-DR (human MHC-II) is elevated on PMNs from frail elderly donors relative to that for young controls, the response to vaccines against pulmonary pathogens declines with frailty (144, 147). It is also possible that elderly donors acquire a T-cell-suppressive phenotype that limits immune responses. Therefore, it is important to understand and consider PMN responses in designing vaccines, since PMNs may provide novel targets for boosting responses in vulnerable elderly individuals.

APPROACHES FOR REVERSING DYSREGULATED PMN RESPONSES IN AGING

PMNs are not the only cells with altered responses during aging; immunosenescence affects other innate immune cells, such as macrophages, NK cells, and DCs, as well as cells of the adaptive arm of the immune response, such as B and T cells (19, 20). Therefore, it is unclear whether targeting deficiencies in PMNs alone would be sufficient to boost host resistance to infections. In proof-of-principle experiments, adoptive transfer of PMNs from young, but not aged, mice prior to pneumococcal infection was sufficient to control bacterial numbers and significantly improve disease scores in aged mice (65). In addition, depletion of PMNs a few hours after IAV challenge ameliorated pulmonary damage and boosted the survival of aged mice (15). These findings do

suggest that approaches that target PMN antimicrobial activity, modulate PMN recruitment, or aid in PMN resolution may be successful in boosting the resistance of aged human hosts to pulmonary infection (Fig. 2).

In order to design successful approaches to correct the age-driven defects in PMN response, a better understanding of what drives these changes during host aging is needed. As discussed above, PMN responses in aged hosts are shaped by several factors. The first are intrinsic changes in PMNs, regardless of the environment (32, 65, 70). Second are local changes in the pulmonary microenvironment that influence PMN recruitment (25, 30, 35, 36, 43, 44) and resolution (15, 25, 30, 41) during infections. Third are the systemic changes in inflammatory cytokines that may alter the phenotype of circulating PMNs (17, 80, 87). Both intrinsic and extrinsic factors should be considered in designing approaches to alter PMN responses.

Targeting inflammaging. The low-grade inflammation that accompanies aging is a result of multiple complex factors (17). These include (i) changes in cellular responses that maintain homeostasis, giving rise to the accumulation of misplaced and misfolded self-molecules, including DNA, proteins, and lipids, that are then recognized by pattern recognition receptors, resulting in cytokine production by innate immune cells, (ii) increases in senescent cells, including, but not limited to, T cells, that display a senescence-associated secretory phenotype (SASP), and (iii) age-driven dysbiosis of the microbiota that contributes to, and is driven by, inflammaging (17). Inflammaging is paradoxically associated with the failure of innate immune cells to respond to acute stimuli. In the context of pulmonary infections, this has been well characterized for macrophages: inflammaging impairs the ability of macrophages to respond to *S. pneumoniae*, resulting in an environment permissive to infection (26, 30). Importantly, blocking inflammatory cytokines, such as TNF-α, reverses the age-driven dysregulation of immune responses to *S. pneumoniae* (148, 149). Cytokine production in response to stimuli is diminished in PMNs from elderly donors (77, 78), and as discussed above, TNF-α may affect PMN responses (87). However, the link between inflammaging and PMNs, and the impact of targeting the inflammatory environment on reversing PMN immunosenescence in elderly donors (Fig. 2), need to be elucidated in further detail.

Targeting PMN signaling. Aging is associated with changes in several PMN signaling pathways (150) (Fig. 2). These include an increase in basal levels of activation of mitogen-activated protein kinase (MAPK) pathways (47, 151, 152) and PI3K signaling (32). These changes render PMNs less responsive to acute stimuli and impair their function. Targeting these pathways has been shown to improve outcomes in elderly donors (Fig. 2). In a model of sterile dermal injury, oral administration of a p38 MAPK inhibitor resulted in enhanced PMN clearance in elderly donors (153). Inhibition of PI3K signaling *ex vivo* restores the accurate migration of PMNs from elderly donors to chemotactic signals (32). Treatment of elderly pneumonia patients with high doses of statins, drugs typically used to control cholesterol levels, also restores the migration accuracy of their PMNs in response to *ex vivo* stimuli (33). This effect was observed only in patients with pneumonia that had not progressed to severe disease, suggesting that statins may be beneficial as an early therapeutic intervention. In fact, administration of a high dose of simvastatin to hospitalized pneumonia patients above the age of 55 years has been shown to improve clinical outcomes, since volunteers who received the statin showed increased hospital-free survival (154), suggesting a reduction in pulmonary damage in these patients.

Mechanistic target of rapamycin (mTOR) is a protein kinase signaling complex that plays an important role in immunosenescence (155). In humans, treatment of elderly donors with a combination of mTOR complex 1 inhibitors boosts Ab responses to influenza vaccines and, importantly, decreases the overall incidence of respiratory tract infections (156). Although the specific incidence of pneumonia, a result of infection of the lower respiratory tract, was not reported in these studies, oral administration of rapamycin, an inhibitor of mTOR complex 1, boosted the survival of aged mice upon pulmonary infection with *S. pneumoniae* (157). While mTOR inhibition has been shown to reverse defects in several types of immune cells (158), whether it has an effect on PMN responses remains unclear.

Aging is also accompanied by upstream changes in G-protein-coupled receptor signaling. Extracellular adenosine, produced by CD73 as discussed above, signals via four G-protein receptors: A1, A2A, A2B, and A3. Age-related changes in EAD signaling (159, 160) have been documented in several organs, including the brain (159), pancreas (161), and heart (160). Recent findings suggest that the EAD pathway also plays a role in PMN immunosenescence. Triggering A1 receptor signaling reverses the age-driven impairment in the ability of PMNs to kill *S. pneumoniae* (65) in mice and, importantly, boosts the resistance of aged mice to pulmonary pneumococcal infection (162). In humans, adenosine-based drugs have historically been used to treat heart arrhythmias (163). However, the effect of adenosine-based interventions on PMN immune responses in aging in human volunteers has not been assessed, likely because full A1 receptor agonists have detrimental effects *in vivo* (163). Therefore, it would be useful to devise and test partial agonists, or prodrugs activated by or targeted to PMNs (164).

Nutritional interventions. Vitamin supplementation is a possible approach to preventing respiratory tract infections (Fig. 2). In several studies, supplementation of elderly subjects with high doses (above the recommended intake) of vitamin D_3 (165) or vitamin E (α-tocopherol) (166) has reduced the risk of upper respiratory tract infections. Although these studies did not specifically assess the incidence of pneumonia, both vitamins D (167) and E (38) boost the ability of neutrophils to kill *S. pneumoniae*. In addition, vitamin E reduces PMN migration across pulmonary epithelial cells in response to pneumococci (38). Importantly, oral supplementation of mice with vitamin E reverses the age-associated susceptibility to *S. pneumoniae* lung infection (25). Note that as a potential nutritional intervention, vitamin intake must be well controlled, since excessive intake leads to toxicity (168).

In conclusion, targeting PMN responses to reverse the enhanced susceptibility of the elderly to pulmonary infection is a viable strategy (Fig. 2), and the search for clinical interventions that target PMNs is ongoing. Such therapies will need to balance boosting PMN function with preventing pulmonary damage by these cells.

SUMMARY AND FUTURE PERSPECTIVES

In conclusion, a clear dysregulation of PMN responses in aged hosts contributes to susceptibility to pulmonary infections (Fig. 1). This dysregulation is manifested by inaccurate PMN migration toward the site of infection, a lack of PMN resolution from the lungs, and impaired PMN antimicrobial function (Fig. 2). These changes are likely driven by systemic inflammaging and alterations in the local lung microenvironment, in addition to intrinsic changes in PMNs. Age-driven dysregulated PMN responses impair the innate control of pathogen numbers and may also play a role in subsequent blunted adaptive immune responses. The mechanism(s) by which dysregulation of innate immunity drives the impairment of adaptive memory responses requires additional study; interventions that target systemic inflammation or PMNs directly may be viable approaches for boosting the resistance of the elderly to pulmonary infection (Fig. 2). A better understanding of the systemic changes as well as the intrinsic signaling pathways controlling PMN responses is needed for the design of improved interventions that boost immunity against pulmonary infections in the growing and vulnerable elderly population.

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